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STUDIES ON FORAGE POISONING*

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I

PATHOLOGIC CHANGES IN A DISEASE IN HORSES RESEMBLING FORAGE POISONING

Previous histo-pathological observations on a disease in horses resembling forage poisoning have been largely confined to the central nervous system.

MacCullum[1] found a softened condition of the anterior region of the brain in gross examination, and in microscopical preparations a complete destruction of the brain substance with colloid degeneration. Congestion was marked, and there were extravasations of the cellular elements of the blood as well as hyaline degeneration of the vessel wall. Moore[2] observed no marked changes in the encephalon. McCarthy and Ravenel[3] described a small round-cell infiltration of the Gasserian ganglia, degenerative changes in the laryngeal nerves, and congestion and capillary hemorrhage of the cerebral and cerebellar cortex. Gross lesions were also observed in the enteron membrane, particularly in the anterior gastro-intestinal tract. Udall[4] in histologic preparations from the hippocampus and the cerebral lobes of the brain found no changes. In more extensive histological examinations in other cases he discovered changes in the olfactory tract and bulb, as well as vascular derangements consisting of lymphocytic infiltrations. Joest and Degen[5] called attention to cell inclusions in the ganglion cells of Ammon's horn when stained with a modified Negri body stain. Mohler[6] noted perivascular round-cell infiltration in the olfactory lobe and the hippocampus. Milks,[7] in 5 cases, noted few changes in the nervous system other than congestion in the meninges. The kidneys, however, disclosed hyperemia and a slight degeneration and desquamation of the tubular epithelium. The liver showed passive congestion. The intestines presented hemorrhages in the mucosa with a thickening of the mucous and submucous layers. No changes were found in the spleen other than a possible increase in white corpuscles. Milks qualified the significance of these changes in view of possible postmortem changes due to climatic conditions.

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[1] Jour. Exper. Med., 1899, 4, p. 409.

[2] Pathology of Infectious Diseases of Animals, 1908, p. 515.

[3] Jour. Med. Research, 1903, 1, p. 243.

[4] Cornell Veterinarian, 1913, p. 17.

[5] Cited by Hutyra and Marek, Pathology and Therapeutics of the Diseases of Domestic Animals, 1912, 2, p. 613.

[6] U. S. Bur. An. Ind., No. 65, 1914.

[7] Bull. Louisiana Agr. Exper. Sta., No. 106, 1908.

With few exceptions the authors consulted have confined their observations to the central nervous system, because the clinical symptoms suggest that the primary focus of the affection is in the central nervous system. A summary of the principal changes described in the literature shows that uniform changes have not been observed. A congested condition of the brain and its appendages has been frequently noted, but no one gross or histologic alteration described can be considered pathognomonic.

In our work the tissues studied were secured from 7 horses fatally affected as the consequence of feeding on an oat hay.[8]. The animals were killed when in a moribund condition, and the organs removed to the laboratory in less than 1 hour after death. These precautions, as well as weather conditions during the early spring of 1915, preclude the possibility of marked postmortem changes.

Specimens were fixed in formalin and in Zenker's fluid, in the usual manner. Sections were stained in hematoxylin-eosin and osmic acid for fat.

Brain.—The brain revealed congestion, the vessels of the various regions of the brain and of the cord being engorged and surrounded by leukocytic and round-cell extravasations. In no region of the cord or brain did we find marked degenerative changes. Cell inclusions, mentioned by Joest and Degen, were not observed.

Heart.—Macroscopically the heart contained a few punctate and ecchymotic hemorrhages in the myocardium, and petechial hemorrhages in the mitral and tricuspid valves. The blood was dark and often coagulated. Microscopically, marked congestion and necrosis of the muscle fibers were observed. An infiltration of endothelial cells accompanied the necrosis.

Lungs.—The gross appearance of the lungs was that of passive congestion, more intense in some cases than in others, with occasional small defined areas of hypostatic congestion. In one case hemorrhagic suggillations were presented, with a few indistinct punctate subpleural hemorrhages. Microscopically the vessels were engorged, but no marked structural or cellular changes were noted.

Stomach and Intestinal Tract.—Gross lesions in the stomach and intestinal tract were not constant, but when observed consisted of small circumscribed areas of hyperemia often accompanied by hemorrhage, a yellowish-white catarrhal exudate adhering to the wall. On the serous surface of the small intestine petechial hemorrhages were sparsely scattered, while in one instance an area of the serous coat of the intestine, approximately 15 cm. in length, was markedly hemorrhagic. In one case the gastric mucosa, and in other cases the intestinal mucosa, was mildly congested in small circumscribed areas. In the cecum and colon the mucosa and submucosa were thickened, and occasionally punctate submucous hemorrhages were seen. The mesenteric lymph nodes appeared slightly swollen and mildly congested in a few cases. Microscopically the mucosa of the stomach showed hemorrhage and exudation, in which polymorphonuclear leukocytes were abundant. The microscopical preparations involving areas of the small and large intestines showed similar changes.

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[8] Graham and Himmelberger: Proceedings of the U. S. Live Stock Sanitary Association, 1915. Jour. Am. Vet. Med. Assn., 1916, 48, p. 574.

Liver.—The liver was in some cases congested. On section small areas of a yellowish color were seen near the margin. Blood often dripped from the cut surface, tho some cases showed no excessive blood content. Histologically the general structure of the liver lobule appeared normal under low magnification, but under higher power, changes of considerable extent were observed. The liver cells were either devoid of nuclei or stained very poorly. At times the degenerative changes were of a fatty nature, the fatty droplets being found within the cytoplasm of the cell and sometimes within the nucleus itself. The cells oftentimes appeared swollen and many lacked nuclei. On the other hand, a superabundance of nuclei appeared to be present in other preparations of this organ, suggesting a reparative process. The capillaries and other vessels were usually engorged, and in the mesh work formed by the capillaries a debris of broken down cells and fragmented nuclei was found. The form of necrosis observed was central. In some of the lobules the necrosis appeared to be on but one side of the central vein and was often complicated by hemorrhage. The blood within the vessels, both central and peripheral, contained large numbers of polymorphonuclear leukocytes.

Kidneys.—The kidneys were congested and in some cases a few minute petechial hemorrhages beneath the capsule were observed in the cortex on section. Microscopically a complicated picture was presented. The disturbance of the glomeruli was extensive. The lining of Bowman's capsule showed a distinct desquamation. The capsular space of some contained both red and white blood cells, a degenerative detritus, and fragmented nuclei. The glomerular tuft was swollen and congested, occupying the capsular space, tho in some cases very little of it remained, the space being occupied to a greater or less extent by a cellular debris. The convoluted tubules showed partial destruction of the tubular epithelium. The epithelial cells were devoid of nuclei; in the lumen of the tubule free nuclei and fragments of degenerated cells could be seen. The capillaries were congested and distended, occasionally obliterating the uriniferous tubules. Within the capillaries there was an accumulation of polymorphonuclear and endothelial leukocytes. The most constant microscopic alterations observed in the 7 cases were those of a hemorrhagic nephritis and a marked injection of the vessels, resulting in the obliteration of adjacent structures.

The gross macroscopic lesions mentioned were not observed in the same degree in each animal, but are referred to collectively, as the various organs were apparently normal in some necropsies. The clinical manifestations in affected animals would have led one to expect extensive changes of more moment, especially in the nervous system. Aside from the congestion noted, the central nervous system was apparently not altered in proportion to the nervous manifestations. According to Adami, some of the most difficult conditions to classify are evidently due to and dependent on circulating toxins which may not produce extensive cellular changes.

The changes in the liver are subject to various interpretations. The congestion may have been due to cardiac or other lesions. The necrosis was of the type considered to be of toxic origin, which includes mineral poisons or bacterial toxins generated within or without the body. Necrotic areas, however, may be found in the liver of apparently healthy animals shortly after death and this fact renders uncertain the exact interpretation of these changes.

The capillary hemorrhage and nephritis may have been the result of any injurious substance exerting an effect on the capillary endothelium. Disturbed heart action and renal function often accompany each other, and in clinical cases a weakened heart action was frequently observed. Cardiac disturbances may also have been partly responsible for the congested condition in the various organs.

The kidney alteration may exercise considerable effect on the bodily health. The lesions observed were not secondary to decumbency, as animals from which tissue was examined were destroyed to avoid such complications. According to von Limbeck,[9] uremia due to disturbed kidney function is the result of retained substances gaining entrance to the circulation.

The gastro-intestinal lesions may have been due to the direct action of poisonous material, or secondary to changes in some other organ, or due to circulating toxins.

While the pathologic presentations in the various tissues examined are not diagnostic, since no pathognomonic importance can be ascribed to them, the changes found collectively are suggestive of a toxemia-like condition in so far as the type of the disease we have studied is concerned.

II

A PATHOGENIC BACILLUS ISOLATED FROM OAT HAY

During the winter of 1914-1915 a disease among horses and mules resembling forage poisoning occurred on the Griffith stock farm in central Kentucky; it was later demonstrated by feeding experiments that of the various feeds on the farm the oat hay was primarily responsible. From the report on feeding experiments with this forage the following summary is quoted:

> "It is evident from these observations that a particular lot of oat hay was responsible for the outbreak of forage poisoning occurring on the Griffith farm and that this disease could be induced in experimental animals (the horse) by the feeding of this material over a period of time which resembled in a measure the incubation period of some infectious animal diseases; also that drinking water contaminated with these oats resulted fatally when given to experimental animals. Other animal species, i. e., guinea-pigs, rabbits, chickens, swine, white mice,

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[9] Arch. f. exper. Path. u. Pharmakol., 1892, 30, p. 180.

goats and sheep, were more resistant and apparently nonsusceptible. It is of interest to note that the oat hay responsible for this disease, tho apparently clean, was later found to be contaminated with the excreta of chickens, which on being fed disguised in wholesome feed resulted fatally. Up to this time, however, we have not been able to locate the causative factor of the disease occurring as a result of feeding this particular lot of oat hay, nor have we been able to show that oat hay from other sources produces forage poisoning in horses and mules."

Much uncertainty exists regarding the cause of forage poisoning; in fact so many etiologic factors have been mentioned that one specific organism cannot be held responsible for all the diseases of horse and mule stock under this caption. In North America moulds of different varieties occurring on vegetation have been incriminated, but feeding experiments with some of the cryptogams isolated from apparently poisonous feed and cultivated under laboratory conditions, suggest only remote connection between some of the moulds referred to in the literature and the disease in question.

Mohler, Mayo, Klimmer, Haslam, and many others observing this disease, consider moulds a possible factor, notwithstanding some of the negative results reported from feeding moulds to experimental horses. A recent bulletin issued by Brown and Ranck of Mississippi[10] describes toxic symptoms and death in guinea-pigs and calves following the injection of the alcoholic extract of the sclerotia of Claviceps paspali and the feeding of the heads of wild grass ("paspalum") infected with Claviceps paspali, and indicates that this fungus possesses toxic properties. It is possible that certain fungi developing in their natural habitat produce poisonous substances which cannot be reproduced under laboratory conditions, but the moulds commonly found on maize and other forage in connection with outbreaks of this disease do not offer a satisfactory solution of the problem. Udahl, Milks, and Williams have suggested that possibly no connection may exist between the feed and so-called forage poisoning.

The oat hay believed to be responsible for the outbreak of forage poisoning at the Griffith farm was not visibly contaminated with mould, but by plating the feed in dilutions, Monascus purpureus (Went) was isolated and artificially cultivated in large quantities for experimental feeding of large and small animals. The results, which have been reported,[11] are summarized as follows:

"*Monascus purpureus* (Went) grown under laboratory conditions had no etiologic significance in this outbreak of forage poisoning, since feeding large quantities over sufficient lengths of time did not produce the disease. We desire to present the evidence of the foregoing experiments as suggestive of the nonpathogenicity of *Monascus purpureus* (Went) when fed in large quantities, and when the products of its metabolism are injected intravenously. *Monascus*

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[10] Tech. Bull. Mississippi Agr. Exper. Sta., No. 6, 1915.

[11] Himmelberger: Jour. Comp. Path. and Therap., 1915.

purpureus isolated from oats which had undoubtedly given rise to forage poisoning in horses and mules did not produce soluble or extracellular toxins *in vitro* on the cultural media employed, as shown by absence of clinical symptoms in the experimental animals."

Among the organisms isolated from the oat hay was a species like B. coli. Rogers, Clark, and Evans recently reported the prevalence of B. coli on American grains.[12] Feeding experiments with horses were conducted with the B. coli-like organisms.[13] No symptoms subsequent to the feeding of large quantities suggested that contamination with B. coli was fatal, or primarily involved in the animal losses, tho mild derangements were produced by feeding the organism disguised in wholesome feed.

Several micro-organisms isolated from the cerebrospinal system of affected animals have been described by many investigators as having an etiologic relation to this class of diseases. Hutyra and Marek[14] summarize these bacteriologic investigations as follows:

"Bacteriological investigations have, therefore, not as yet given perfectly satisfactory results, although it is probable that Siedamgrotzky and Schlegel, Johne, Ostertag, Streit, Grimm, Christiani, Marcq, and possibly also Wilson and Brimhall, were dealing with the same organism which had in some way varied its characters somewhat. Further investigations are necessary to decide whether the cause of so-called Borna disease is always present in cases of cerebrospinal meningitis in the horse, and whether it plays any part in the production of the disease in other species, at least in a proportion of cases. The observations of Prietsch, Walther, Pröger, and Wilson and Brimhall, appear to indicate that this is the case. A solution is also required to the question as to what relationship exists between the organism described by Johne and others and the diplococcus intracellularis of the human subject. According to Johne the two may be distinguished by the fact that the organism which occurs in the horse may be present in the central nervous system without causing lesions, but simply an intoxication. According to Ostertag there is no connection between the two organisms. Christiani, on the other hand, was unable to find any differences between the streptococcus found by him and the diplococcus intracellularis of Weichselbaum."

Reichel informed us of a pathogenic coccus-like organism isolated from the cerebrospinal system of a horse that suffered from a disease resembling forage poisoning, as well as from a nonaffected horse that had been destroyed for student anatomic exercises. Our own efforts to isolate a pathogenic micro-organism from the encephalon of animals, naturally and experimentally affected, were not successful.

From the oat hay which formed the basis of our experiments a pathogenic micro-organism was isolated which grew readily under laboratory conditions. A somewhat similar micro-organism has been isolated from the chicken excreta found in the oat hay, but its pathogenicity has not yet been established. This micro-organism was not isolated from 2 apparently wholesome forages examined bacteriologically, but from sorghum ensilage obtained from a farm where animal fatalities had occurred with symptoms resembling forage poisoning, a micro-organism with somewhat similar cultural characteristics was obtained. The micro-organism from the sorghum ensilage possesses pathogenic properties, as observed following inoculation of experimental horses.

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[12] Jour. Infect. Dis., 1915, 17, p. 137.

[13] Graham and Himmelberger: Jour. Bacteriol., 1915, 1, p. 115.

[14] Pathology and Therapeutics of the Diseases of Domestic Animals, 1913, 2, p. 610.

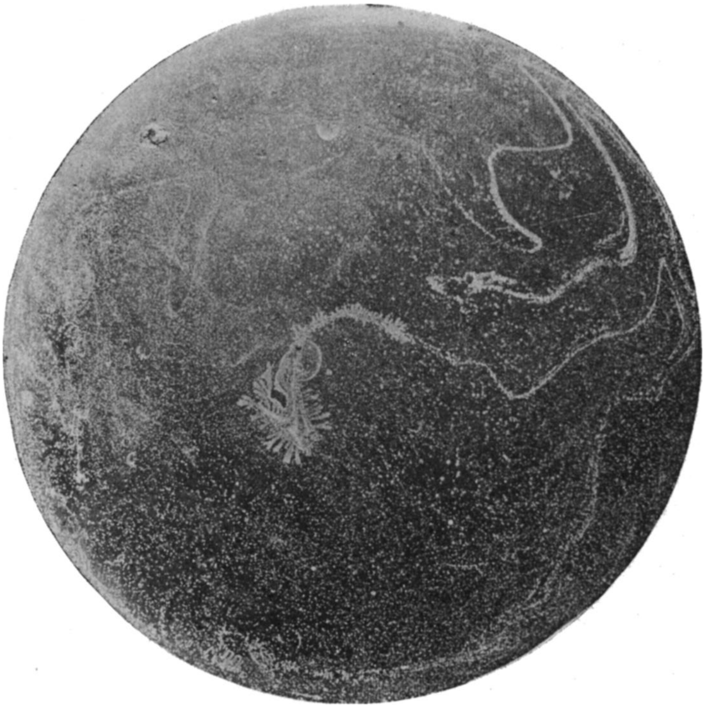

Fig. 1. Poured agar plate of the bacillus isolated from oat hay (Culture O^1 P^2, 4 days old).

The micro-organism isolated from the oat hay exhibited the following cultural* and pathogenic characteristics:

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* The cultural characters here described were corroborated by Prof. R. E. Buchanan of the Iowa State College and Dr. I. J. Kligler of the American Museum of Natural History, working with subcultures submitted by the authors.

MORPHOLOGY AND STAINING PROPERTIES

It is a bacillus, from 0.4 to 0.5 micron wide and from 1 to 2 microns long, occasionally longer, with rounded ends. In newly isolated cultures it resembles a coccoid bacillus. It occurs singly, chains of 2 bacilli being rarely found. It is motile, possessing flagella. Spores are produced, as demonstrated by heating a broth culture to 80 C. for 15 minutes and subculturing, as well as by staining reactions. It is stained by the ordinary aniline dyes, tho often unevenly with a tendency to grouping in the field. Gram-negative. The optimal temperature is from 35 to 37 C. It is aerobic, satisfactory growths not being obtained under strictly anaerobic conditions.

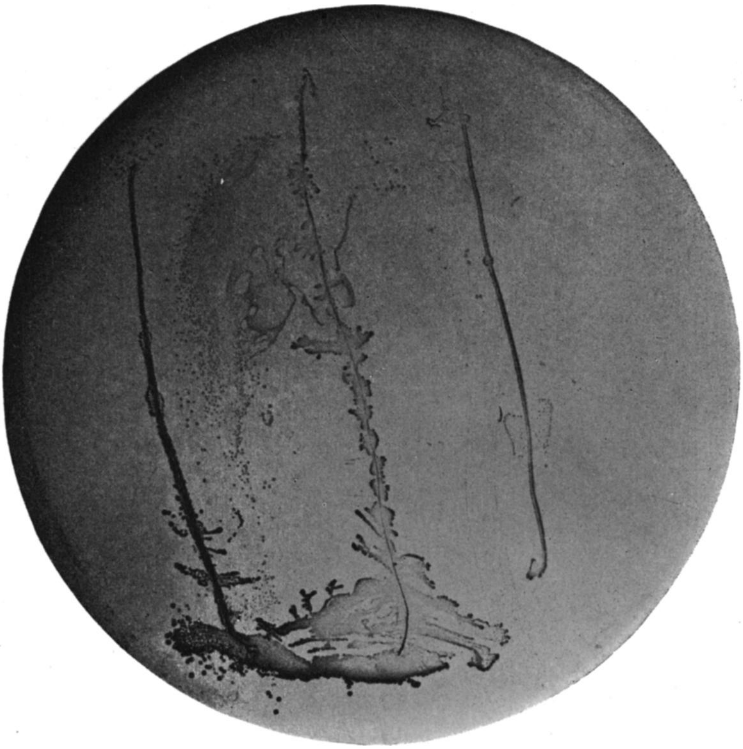

Fig. 2. Stroke cultures on an agar plate of the bacillus isolated from oat hay (Culture O^1 P^2).

CULTURAL CHARACTERISTICS

Agar Plate.—Small bluish-white colonies make their appearance in 24 hours as surface and frequently as sub-surface colonies in poured plates. Surface colonies increase in diameter after 2 or 3 days' incubation, and are oval or round, occasionally spreading over the surface. Rosette figures may be observed. Sub-surface colonies remain small. Under magnification of 15 diameters they are of a yellowish amber color. Not sensitive to variations in alkalinity or acidity of culture medium.

Agar Slant.—A thin growth along the line of inoculation is visible in 24 hours; isolated colonies at a distance from the line of inoculation are also common. As the growth becomes heavier it may show echinulate formations along the line of inoculation, more marked at the base. A white or amber tinge is observed in older cultures, and the growth may be continuous and compact, with a wrinkled surface. Growth in young cultures is of a butyrous-like consistency. An unpleasant odor is sometimes detected in old cultures.

Gelatin Stab.—Growth on the surface is abundant, developing faintly along the line of inoculation. Slight liquefaction observed after 1 week.

Litmus Milk.—Peptonization.

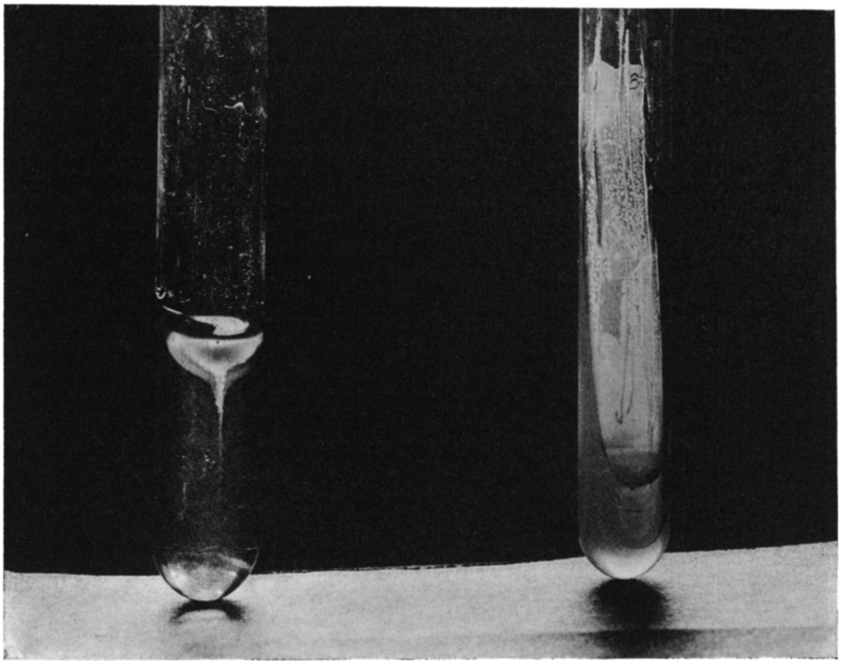

Fig. 3. Tube on left: Gelatin stab of the bacillus isolated from oat hay (O^1 P^2) 10 days old. Tube on right: Agar-slant culture (O^1 P^2) 1 week old.

Gas-Production in Sugar Broth.—No gas formed in maltose, raffinose, saccharose, rhamnose, dextrose, inulin, lactose, and mannite broth. No appreciable acid formed.

Indol and Nitrite-Production.—Indol-production could not be detected in Dunham's solution. Nitrates not reduced.

PATHOGENESIS

The bacillus is apparently nonpathogenic for rabbits, guinea-pigs, white rats, chickens, cats, dogs, and domestic swine, as observed from feeding and inoculation experiments to date. As very few of the large family of aerobic spore-forming bacteria have heretofore proved pathogenic, this organism was for a time disregarded in our studies. It was later observed that horses, mules, cattle, sheep, and goats do not succumb to a single intravenous injection except in rare instances, tho symptoms of a transitory nature are generally observed, such as muscular tremors, dull, sleepy attitude, altered respiration. The growth on agar slants administered in sterile salt solution intravenously to horses results in marked symptoms in from 5 to 30 minutes. Repeated daily injections in horses are followed by nervous manifestations, marasmus, coma, and death. Cattle, sheep, and goats are more tolerant of similar injections, and some horses more so than others.

Broth cultures of the bacillus administered daily in doses of from 500 to 1,500 c.c. to horses by way of the mouth cause death in from 10 to 12 days. Beginning on or about the 5th day marked symptoms are observed from day to day, subsequent to each drenching, of muscular tremors, altered respiration, difficult deglutition, salivation, local paralysis, diffuse sweating, marasmus, prostration, and death. Broth cultures administered daily in 1,000 c.c. doses in the form of an enema to horses receiving wholesome feed cause prostration and death in from 7 to 12 days.

Cultures grown on Uschinsky's medium after passing through sterile cotton filters cause marked symptoms of distress when injected intravenously. Daily intrajugular injections of cultures obtained by filtration through Pasteur-Chamberland "F" and "B" candles, and sub-cultured to determine their sterility, result fatally in from 3 to 15 days. The symptoms manifested by animals so treated consist of a general depression, muscular tremor, salivation, difficult mastication, constipation, marasmus, incoordination of voluntary muscles, and inability to stand. While in a recumbent position the animals move their feet as if walking, or lapse into a condition of coma; during this time an indifferent nervous appetite may accompany a paretic-like condition of the pharynx. Death generally follows in from 1 to 5 days after the animal becomes unable to stand.

Passing through Pasteur-Chamberland "F" and "B" candles apparently modifies the virulence of the filtrate, tho heating to 80 C. in a hot bath does not destroy its effect entirely, as observed in animal inoculation. An active culture filtrate was tested chemically for protein by Dr. G. D. Buckner of the experiment station, with negative results, further confirmed by the absence of anaphylactic phenomena subsequent to guinea-pig and rabbit inoculations.

The symptoms observed in experimental horses following the injection of the sterile filtrate of this bacillus grown on Uschinsky's medium evidence the production of a toxin-like substance as an inherent character of the bacillus isolated from the oat hay, and the results from repeated administrations suggest a systemic cumulative action.

Repeated intravenous injections of the bacillus washed from agar slants, sterile filtrates of the culture on Uschinsky's medium, broth cultures by way of the mouth or in the form of enemas produced variable nervous symptoms and death in horses and mules. The fact that the oat hay, fatal when fed to horses, produced no effect when fed to small laboratory animals is in accord with the existing knowledge of forage poisoning; the nonpathogenicity for small animals of the bacillus here described therefore contributes to its significance as a pathogenic factor in this disease.